Creosote P1/P13: 90-Day Dermal Toxicity in Rats.
Creosote Council. 1993. MRID No. 43616101

Government of Canada / Gouvernement du Canada

MEMORANDUM **NOTE DE SERVICE**

TO / Ä: Don Grant, Director
Health Evaluation Division
PMRA

FROM / DE: Anna Harper, Evaluator
Non-Food Toxicology Section
Health Evaluation Division
PMRA

SECURITY - CLASSIFICATION - DE SÉCURITÉ

OUR FILE - N / RÉFÉRENCE
SUB # H95001400

YOUR FILE - V / RÉFÉRENCE

DATE
July 18 , 1996

SUBJECT / OBJET: **Reevaluation**
Creosote - 90 Day Dermal Toxicity Study with P1/P13 mixture

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Test Article:	North American P1/P13 Creosote CTM
Performing Laboratory:	International Research and Development Corp. (IRDC) 500 North Main St. Mattawan, MI 49071
Project Identification:	IRDC 671-013
Sponsor:	The Creosote Council II
Project Report:	April 13, 1995

EPA Reviewer: Tim McMahon, Ph.D._____ Date: _____
Senior Scientist, RASSB/AD (7510C)

DATA EVALUATION RECORD

Title: R.A. Hilaski; April 13, 1995; **North American P1/P13 Creosote CTM: 90-Day Subchronic Dermal Toxicity Study In Rats.** IRDC, Mattawan, MI.; Report No. 671-013. Sponsored by The Creosote Council II. MRID # 43616101 Unpublished. Study I.D. : IRDC 671-013

Performing Lab: IRDC, Mattawan, MI

Test article: North American P1/P13 Creosote CTM was received from North American Creosote. It was described as a black liquid and a composite mixture and assigned an IRDC number of 10960B. The vehicle, corn oil, was received from Bio Serv and Hunt Wesson Inc. It was described as a yellow liquid of unknown purity. Appendix A of the submission describes a method for the determination of percent of the nine most prevalent compounds in creosote, however, no information is given as to the identity or relative percentages of these nine compounds in the final composite mixture. The test article material was prepared weekly in corn oil and stored in amber glass bottles at room temperature. For the control group, corn oil was stored in amber glass bottles as well.
Analyses of test article stability and concentration in dosing solutions were carried out. It was found that dosage mixtures held for 10 days contained 88 to 108% of the initial "day 0" test article concentrations and, therefore, were stable under the selected storage conditions. Test mixtures administered during weeks 1-4, 8 and 12 contained 95 to 108% of the desired concentrations of the test article.

Test System: Seven-week old male and female Charles River Crl:CD BR rats were obtained from Charles River Laboratories, Portage, Michigan. Rats were acclimated for 8 days prior to study initiation. They were individually housed in animal rooms with controlled temperature (72°F), relative humidity (43%) and light cycle (12 hours light: 12 hours dark). Food and water were available ad libitum. Forty males (weighing from 242 to 269 g) and forty females (weighing from 182 to 202 g) were randomly selected and assigned to treatment or control groups.

Dose levels: The test article and vehicle were administered dermally at dosage levels of 4, 40 or 400 mg/kg bw at a volume of 2 ml/kg bw once daily, five days a week, throughout the 90 day study. The control animals received the corn oil vehicle at a dosage volume comparable to that received by the test animals.

The dosage levels utilized in this study were selected on the basis of available data from a previous IRDC range-finding study 671-024 **"Two Week Dermal Study of North American P1/P13 Creosote in Rats"**. In this study 3 rats/sex/dose were exposed to 0, 3, 10, 300, 1000 (mixed in corn oil) or 2000 (undiluted) mg/kg bw for 6 hours per day for 14 days. Dermal irritation was assessed according to Draize. No dermal irritation was observed at dose levels of 3, 10 and 300 mg/kg bw. Dermal irritation in the 1000 mg/kg bw group was limited to slight erythema in 1 to 3 rats at any given time point after study day 8. In the highest dose group of 2000 mg/kg bw slight to moderate erythema (3-5 rats) and slight edema (3-6 rats) was observed initially at study days 5 and 7, respectively. These signs persisted through to

study termination. Desquamation was observed in 2 rats beginning on the seventh day of exposure. Based on these findings a top dose level of 400 mg/kg bw was recommended for the definitive 90-day dermal irritation study.

Additional correspondence from J. Butala, representing the Creosote Council (Feb. 14, 1996) supported this dose level selection. He stated that experience with somewhat similar petroleum distillates suggested that repeat dermal application produced no or little irritation initially and then, after the first five days or so of application, caused a dramatic increase in dermal irritation. Results from the range-finding studies with these test creosotes (P1/13 and P2) paralleled the petroleum observations. To ensure that the definitive studies would reach the full 90 days, top dose levels of 400 mg/kg bw were selected.

Treatment: Treatment groups of 10 rats/sex/dose were used in this study. The animals were dosed once daily for a six hour exposure period, five days per week for 13 weeks (a total of 65 daily applications). Prior to initial dosing the dorsal skin (approx. 15% of body surface) of each rat was closely clipped free of hair using an electric clipper. The test mixture or corn oil alone was applied to approximately 10% of each rat's total body surface. The application area was clipped throughout the study period as necessary. The prepared test mixture was evenly distributed over the prescribed area using a glass stirring rod. After each application, the treated area was wrapped with gauze bandaging and non-irritating tape. Following the exposure period, the bandaging materials were removed and the treated area was washed with tepid tap water.

Observations for mortality, morbidity or reaction to treatment were made at least twice daily throughout the study. More thorough physical examinations were made weekly at the time of weighing. Body weight and feed consumption were made at pretest and weekly during the course of the study. Ophthalmoscopic examinations were made during the pretest period and during the last week of the study. Examination of the cornea, conjunctiva, sclera, iris and fundus was performed following pupillary dilatation with 1% tropicamide solution.

Skin irritation was assessed on each treatment day before the application of the test material and was scored on the Draize scale.

Clinical laboratory tests were conducted on all surviving animals at termination. Blood samples were obtained from the orbital sinus following an overnight fasting period. Urine was collected during the fasting period over a collection period of approximately 16 hours.
Hematological parameters assessed: leukocyte count, erythrocyte count, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, differential leukocyte count.
Biochemical parameters assessed: concentrations of sodium, potassium, chloride, calcium, inorganic phosphorous, total bilirubin, urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, serum cholesterol, glucose and activities of alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic dehydrogenase (LDH), creatine, phosphokinase (CPK).
Urinalysis parameters assessed: colour, appearance, volume, specific gravity, microscopic elements, osmolality, pH, protein, glucose, ketones, bilirubin, occult blood, nitrite, urobilinogen, leukocytes.

Any animals found dead during the course of the study were necropsied. At terminal

sacrifice all remaining animals were euthanized by carbon dioxide asphyxiation and subjected to a complete macroscopic postmortem examination.

The following organ weights were determined: brain, testes, heart, kidney and liver. Representative samples of protocol-designated organs and tissues were collected from all animals and placed in phosphate-buffered neutral formalin for microscopic examination: adrenals, aorta, bone with bone marrow, bone marrow smear, brain, eye, optic nerve, gastrointestinal tract, ovary, testis with epididymis, heart, kidney, lacrimal gland, liver, lung with bronchi, lymph nodes, mammary glands, oviduct, pancreas, pituitary, prostate and seminal vesicles, salivary gland, sciatic nerve, skeletal muscle, skin treated and untreated, spinal cord, spleen, sternum, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, cervix and all gross lesions.

All of the tissues/organs listed above were examined microscopically from all animals in the control and 400 mg/kg bw group. Only sections of heart, liver, kidney, lung and any gross lesions were examined from animals of the 4 and 40 mg/kg bw groups. A grading system of trace, mild, moderate and severe was used to define lesions for comparison between dosage groups. Appropriate statistical analyses were performed.

A formal peer review of the histopathologic findings was performed. This review included all tissues and diagnoses from 2 animals/sex in the control group and 6 animals/sex in the 400 mg/kg bw group.

Results:

Mortality: One male rat of the 400 mg/kg bw group (#46166) was found dead on day 79 of the study. It had slight erythema of the treatment area on days 7, 8, 9, 25, 26, 31, 32 and a scabbed area on the left shoulder area during week 5. Necropsy and histopathological examination revealed no explanation as to the early death of this animal. All other animals survived to termination.

Clinical Signs: Scabbed areas on the dorsal skin surface (shoulder areas and head, not graded) were seen in all male groups and in control and in the 4 and 40 mg/kg bw female groups throughout the study and, therefore, are not considered to be treatment-related. Other infrequent clinical observations which included discoloured urine, bruises and eye abnormalities were not considered to be due to the application of the test article.

Body Weight: No treatment-related effects were observed in any animals of any group as shown in the following table:

MEAN BODY WEIGHT (gm ± SD)

GROUPS (n=10)	SEX	WEEK 1	WEEK 6	WEEK 13
Control	male	253 ± 7.3	461 ± 27.9	535 ± 43.9
4 mg/kg bw	"	253 ± 7.6	461 ± 32.8	531 ± 45.6
40 mg/kg bw	"	254 ± 7.8	457 ± 18.0	522 ± 31.7
400 mg/kg bw	"	254 ± 8.2	465 ± 36.5	543 ± 37.6
Control	female	192 ± 5.9	244 ± 12.4	259 ± 15.5
4 mg/kg bw	"	191 ± 5.6	245 ± 12.2	259 ± 16.0
40 mg/kg bw	"	191 ± 5.8	247 ± 19.6	263 ± 21.8
400 mg/kg bw	"	191 ± 6.0	250 ± 17.5	266 ± 17.5

Food Consumption: No treatment-related effects were observed in any groups.

Dermal Irritation: Dermal irritation was observed in control and all treated groups as shown in the following table:

ERYTHEMA

Group (n=20) Combined sexes*	No. Animals with Erythema	Duration of Erythema
Control	5/20 (5f)	Slight erythema Days 4 to 7
4 mg/kg bw	2/20 (1m/1f)	Slight erythema Days 4 to 11
40 mg/kg bw	none	
400 mg/kg bw	6/20 (1m/5f)	Slight erythema Days 3 to 9 and sporadically to day 32

DESQUAMATION	No. Animals with desquamation	Duration of Desquamation
Control	5/20 (5f)	Days 4 to 10
4 mg/kg bw	2/20 (2f)	Days 4 to 11
40 mg/kg bw	2/20 (2f)	Days 3 to 9
400 mg/kg bw	3/20 (3f)	Days 7 to 11

* m = male/ f = female
"Slight" erythema was defined by the authors to be well defined erythema.
"Desquamation" was scored as "yes" or "no" indicating its presence or absence.

Ophthalmoscopic Examination: Two male rats (1 control and one 400 mg/kg bw dose group) and 1 female rat (40 mg/kg bw dose group) had chorioretinal hypoplasia in the right eye. These observations were not considered to be treatment-related.

Hematology/ Urinalysis: No treatment-related changes were observed in any of the parameters.

Clinical Chemistry: Potassium levels were slightly decreased (5%) in the males of the 4 and 40 mg/kg bw groups. Although these differences are statistically significant ($P<0.05$) they are of questionable biological significance in this study since no animals in the 400 mg/kg bw group had decreased potassium levels.

Organ Weights: There were no significant treatment-related effects on organ weights in this study.

Pathology: Gross and histopathological examinations revealed no treatment-related dermal or systemic pathology in any group. Minor skin changes (trace, focal chronic inflammation; mild, focal acanthosis) were noted in a few animals across all groups. There were no test article-related microscopic lesions in the application site on the skin or in any other organs or tissues examined from any rats in any of the treated groups.
No explanation of the grading system for histopathological lesions was included in the submission.
Peer reviewer was in agreement with author's conclusions regarding the histopathological assessment.

Author's Conclusion: Based upon the death of one male in the 400 mg/kg bw group only, the No Observed Effect Level (NOEL) in males for North American P1/P13 Creosote CTM in this study was 40 mg/kg bw. Since no adverse or test article-related findings were detected or observed for any females, the NOEL for females for P1/P13 Creosote CTM is greater than 400 mg/kg bw.

Reviewer's Conclusion: Since there was no treatment-related pathology data (either dermal or systemic) to explain the early death (day 79/92) of the 400 mg/kg bw group male rat #46166, the reviewer believes that the NOEL should be set at 400 mg/kg bw for male rats, as well as for female rats. Female rats may be more dermally sensitive to the application of creosote since they exhibited a greater incidence of dermal irritation across all groups than did the male rats. It seemed unusual that corn oil was used as the vehicle for administering the test article and that very little dermal irritation was evident for a test material that is a known irritant. The registrant was asked to provide a rationale for the use of corn oil in this study.

In response to our query on the use of corn oil as the vehicle in this study, J. Butala, representing the Creosote Council (letter of Feb. 14, 1996), stated that it was their belief that undiluted creosote was too irritating to be applied repeatedly to the test animal's skin. Corn oil was selected as the vehicle based on the following reasons:

1. Very low potential for dermal or systemic toxicity;
2. Absence of interfering contaminants;
3. Solubility of creosote in corn oil;
4. Long experience with corn oil as a vehicle for oral studies;
5. Near universal employment of corn oil as a vehicle for lipophilic test materials in dermal toxicity studies;
6. Validated analytical methodology was available for corn oil-creosote mixtures.

This rationale was accepted.

Executive Summary: In a 90-day dermal toxicity study (MRID # 43616101), 10 Charels River Crl:CD BR rats (10/sex/dose) were given dermal applications of P1/P13 creosote in corn oil mixture at dosage levels of 0, 4, 40 or 400 mg/kg bw/day. There were no treatment-related effects from dermal application of P1/P13 creosote on body weight, food consumption, ophthalmology, hematology, clinical chemistry, or organ weights at any dose level tested. Mortality (death of one male rat at 400 mg/kg/day) was observed on day 79 of the study. No test-article related microscpoic lesions were noted at the application site on the skin at any dose level. Based on the results of this study, the systemic LOAEL was determined to be 400 mg/kg bw/day for both male and female rats, based on mortality. The systemic NOAEL was determined to be 40 mg/kg/day.

This study is classified as **acceptable** and satisfies the guideline requirement (OPPTS 870.3250; OPP 82-3) for a subchronic dermal toxicity study in rats for P1/P13 creosote.